NCCT Project Descriptions
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Project Title: The Virtual Embryo Project: A Computational Framework for Developmental Toxicity

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Key Participants: Imran Shah, Richard Judson, Amar Singh (Lockheed Martin), postdoctoral fellow (*To Be Named*), and graduate student or undergraduate intern. Because this is a new project (September, 2007) the key NCCT participants are not yet identified. Initial discussion involved Bob Kavlock, Jerry Blancato, David Dix, Ann Richard and others in the Biological Modeling Group.

1. Short description of the topic/project

The goal of this new project is to advance EPA's Computational Toxicology program by building capacity for predictive modeling and regulatory analysis of developmental processes and toxicities. Computational techniques fall into two general branches: knowledge-discovery (data-collection, mining and analysis) and computer simulation (mathematical modeling at various levels of detail). Our hypothesis states that critical effects of environmental agents on embryonic tissues can be captured from computer simulations of normal development that draw from an open-source toolbox addressing knowledge regarding the flow of molecular regulatory information in rudimentary tissues, the cell-autonomous responses to genetic (programmed) and environmental (induced) signals, and the emergent morphogenetic properties associated with collective cellular behavior in any given system.

For proof of principle, The Virtual Embryo Project (*v-Embryo*) will address early eye development. This morphogenetic system has well-studied anatomical landmarks and developmental phenotypes. It requires precisely-timed interactions between surface ectoderm and forebrain neuroepithelium that give rise to the lens and cornea (surface ectoderm), pigmented retina and sensory retina (neural ectoderm), and iris and ciliary body (neural crest). The key genes and signaling pathways orchestrating these inductive phenomena are highly conserved. As such, eye development is simple enough to capture a great deal of experimental detail for computer simulation and yet complex enough to warrant the engineering of a computational toolbox that can be applied to analyze critical events *in silico*, thus enabling predictive models for developmental toxicity. That said, the short-term (1-2 yr) objective of this project is to build a directed computer animation and accompanying knowledge management system of vertebrate eye development that is biologically-based and mathematically-driven. The prototype will be subsequently validated / refined against new and existing data from standardized tests during the intermediate period (3-5 yr). Over the interim we will also begin to consider other embryological systems and concepts to expand the scientific capability and regulatory utility of *v-Embryo*. This includes designing a 'computational toolbox' for our Birth Defects Systems Manager (BDSM) domain that will be implemented on ACToR. Such a toolbox will enhance basic research efforts for the EPA and the broader scientific community as a novel platform for integrative embryology. Over the long-term this platform may be used to predict how the embryo might react to low-dose chemical exposure, at different stages, and across species. The *v-Embryo* will align closely with other NCCT initiatives in chemical prioritization and dose extrapolation through the ToxCast^{TM} Research Program and the Virtual Liver Project.

2. What is the EPA context for the project?

The potential of an environmental chemical to cause adverse effects in the developing embryo or fetus is an important consideration in any health risk assessment. Such information is usually derived from experimental studies in which pregnant laboratory animals are exposed to various concentrations of

compounds during critical stages of fetal development. Many EPA risk assessments have selected developmental endpoints (rather than cancer) for a critical effect. For example, sampling of 43 toxicological reviews in NCEA's Integrated Risk Information System (http://www.epa.gov/iris) assessment database showed that 12 cases (28%) had critical effects selected for childhood or gender susceptibilities [S Euling, personal communication]. These endpoints included altered birth weight, skeletal variations and neurodevelopmental defects, altered maternal or postnatal weight gains in reproductive organs, and testicular lesions.

One might therefore expect for such cases where adverse effects-data are available following early life exposure that certain developmental endpoints would be critically sensitive to lower doses. Because many chemicals have data gaps for early life exposures, and because reproductive-developmental testing is the largest consumer of animal resources [Bremer et al. 2007], one place where the NCCT's work could make a real difference is predicting toxicity after developmental exposure. Especially if such computer simulations would integrate vast amounts of mechanistic data to lower the high false positive rates that plague alternative testing strategies, then this would greatly aid risk assessment by improving chemical prioritization for animal testing [National Research Council, 2007]. Accurately predicting dose-dependent developmental toxicity will help risk assessment by prioritizing animal testing for environmental chemicals by enabling 'what-if' kinds of experiments to systematically test hypotheses for agent-based outcomes that cycle through data-driven mechanisms and mode of action.

3. What are the strategic directions and science challenges?

Morphogenesis is a tissue phenomenon. Bard's developmental ontology [Bard, 2007] is predicated on 4 core processes: (1) *patterning*, which sets up future events in cellular subpopulation; (2) *proliferation and apoptosis* as the basis of growth and shaping; (3) *cell differentiation*, which refers to the changing of cellular phenotype; and (4) *morphogenesis* as the generation of spatial organization (e.g., movements). Each core process has a number of subprocesses that are archived in the 'biological process' namespace of Gene Ontology. Successful modeling must address programmed (genetic) and inducible (environmental) changes to core processes and subprocesses that unfold by the hour. Incorporating this level of biological detail into risk assessment will be a non-trivial task. One issue is that the framework data reside in a highly distributed system of literature, databases, and paper records. Building capacity for biological modeling and regulatory analysis thus requires an information infrastructure to enable knowledge-discovery (data-collection, mining and analysis) and simulation (mathematical modeling at various levels of detail). The need for functional models to analyze and visualize critical steps leading to abnormal morphogenesis speaks to a second key challenge for science and technology, that being a 'computational toolbox' with the capability to integrate heterogeneous data into biological networks to model emergent properties of a developing system.

Our initial effort in this regard will focus on early eye development since there is reasonable amount of knowledge available. Eye development can be perturbed by genetic mutations and environmental exposures, leading to malformations such as anophthalmia, microphthalmia, colobomata, and cataract. These defects occur in more than a million children worldwide (6.8 per 10,000 live births, ~28,000 annually in the US). An OVID search of the Medline database revealed specific reference to ocular malformations in 25% of mouse teratology literature. This implies broad susceptibility of the eye to diverse agents. Teratogens that perturb mitochondrial function, for example, almost invariably perturb early eye development [Wubah et al. 2001; O'Hara et al. 2002; O'Hara et al. 2003; Charlap et al. 2003; Green et al. 2007]. The major challenge for technology development will be to transform such data-driven resources into computational models and visualization tools that enable questions regarding: how individual cells collectively influence emergent properties at higher levels of organization; which modeling framework(s) best capture the cellular changes in time and space; what insightful predictions can be made about mode-of-action for specific agents; and what are the characteristics of the dose-

response relationship. Through systematic 'parameter sweeps' we can begin to define associative relationships in cellular-molecular characteristics, identify moments in time at which interventions have extreme consequences ('lever points'), and distinguish among types of path dependency leading to structural and functional abnormalities. Thus, computational systems-based analysis can greatly facilitate research to characterize biological thresholds in developmental toxicity.

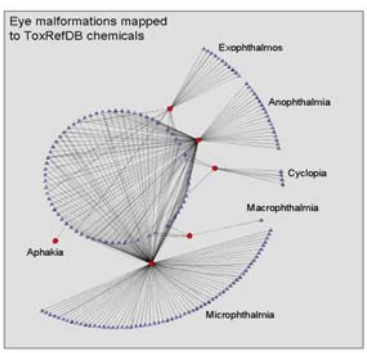
4. What are the short-term (1-2 year) and long-term (3-5 year) goals?

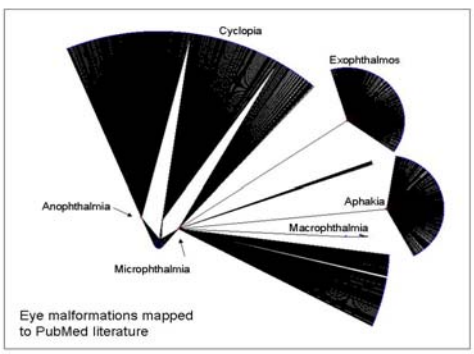
4.a. Knowledge-discovery: building information infrastructure for biological modeling and regulatory analysis of developmental processes and toxicities

v-Embryo will implement a new resource in ACToR previously referred to as Birth Defects Systems Manager (BDSM). Developed at the University of Louisville by Knudsen and Singh, BDSM provides an integrative knowledge management system. At its core is a comprehensive reference collection of gene-expression data, software applications and tools to link these data to user-defined embryological and/or teratological features. A high-performance computing infrastructure and custom tools are available to mine these data and derive gene-expression signatures [Singh et al. 2005; Knudsen et al. 2005; Singh et al. 2007a and 2007b]. The short-term goals are to: build a searchable document repository; implement a semi-automated data-capture routine that reaches out to databases and resources using a thesaurus of metadata terms; develop a Natural Language Processing (NLP) lexicon that flags records holding developmental data and extracts content using logic-based text mining rules; and create a *Regulatory Toxicology Support System* (RTSS) to facilitate research translation.

For example, ToxRefDB is the reference database anchors ToxCast™ to traditional toxicology endpoints. It was built from 4618 Data Evaluation Records (DERs) held at the Office of Pesticide Programs in EPA Program Offices, representing well-studied chemicals that make up a large share (280) of the ~320 chemicals submitted for Phase-I. Although ToxRefDB has already been manually curated for general developmental endpoints [Matt Martin, NCCT], the large number of DERs even larger body of scientific literature presents challenges for mining keyword combinations. Commercial software (http://www.edocfile.com/tifftotext/identifydocs.htm) and custom Perl scripts will utilize Microsoft's optical character recognition (OCR) engine to build a domain repository of searchable text files for text-mining. The shallow parser (SearchDER.pl) will output an *interactive matrix for document classification* based on an input list of user-defined keywords from a thesaurus of 984 metadata terms (http://www.devtox.org) for fetal abnormalities.

A potential use of logic-based text-mining is finding chemical relations by effects-description, target, and keyword combinations. For example, consider the 37 DevTox terms associated with eye malformations. One can rapidly visualize relationships for document classification and feature mining by mapping associative relationships with Cytoscape 2.5.1 (http://www.cytoscape.org/features.php) [Shannon et al. 2003]. In this way a single chemical may connect with multiple keywords or multiple chemicals may connect to a single keyword. A chemical that did not have a keyword from the list would not be included in this diagram; nor would a keyword that was not found in the DER library be displayed. Overall, 160 chemicals and 6 ocular conditions were captured from the DER records. Searching PubMed by the 160 chemicals returns 13,027 PMIDs of





which 2,282 refer to the eye. The obvious challenge is to limit linkages to those with important facts. This requires a NLP lexicon built from learned patterns of text and meta-data. As a specific example, consider:

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<observation>: “over expression of” | “under expression of” | “co-regulation of” |
<gene>: “PKA” | “PKB” | “PCNS” | “RAP” | (any gene related to development) |
<stage or location>: “in the liver” | “in gastrulation” | “during gastrulation” |
<effect>: “causes” | “results in” | “activates” | “controls” | “regulates” |
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When a regular expression parser is applied to abstracts available in PubMed, entries such as the following excerpt is flagged as potentially important:

“… Overexpression of PCNS resulted in gastrulation failure but conferred little if any specific adhesion on ectodermal cells. Loss of function accomplished independently with two non-overlapping antisense morpholino oligonucleotides resulted in failure of CNC migration, leading to severe defects in the craniofacial skeleton. …” (Rangarajan J et al., Dev Biol 2006, 295(1):206-218. PMID: 16674935)

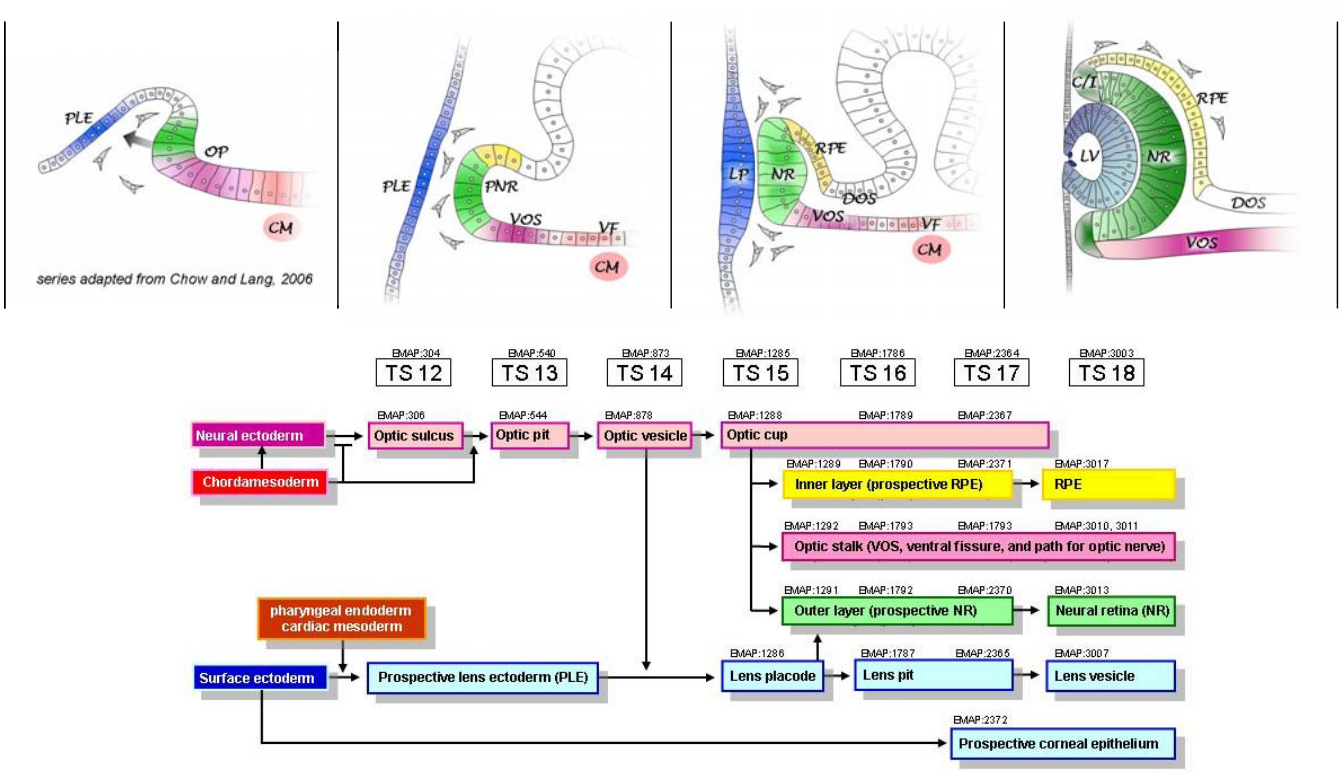
We will carry these text-mining tools forward to build the genetic circuitry of eye development (1-2 yrs) using BioTapestry (http://www.biotapestry.org) software [Longabaugh et al. 2005] that meets the informational needs for computational modeling of developmental gene regulatory networks using the SBML workbench [Hucka et al. 2003]. Assuming logic-based text-mining gains regulatory acceptance for assessing developmental endpoints, the *Regulatory Toxicology Support System (RTSS)* will offer NCEA stakeholders and program managers a critical performance appraisal of the computational techniques developed in *v-Embryo.* RTSS will address the basic science foundation for normal embryology (*embryo-formatics*), a prioritization schema to maintain focus on research to support risk assessment, strategic planning of specific case examples to calibrate the system, and finally improved ways to translate the work more effectively.

4.b. Computer simulation: *In silico* models of organ system (eye) development

To calibrate the framework and build useful content onto BDSM, we need bottom-up (forward) models of development. An idealized model would parameterize chemical mode of action and accurately simulate phenotype outcome. Our hypothesis states that critical effects of environmental agents on embryonic tissues can be captured from computer simulations of normal development that draw from an open-source toolbox addressing knowledge regarding the flow of molecular regulatory information in rudimentary tissues, the cell-autonomous responses to genetic (programmed) and environmental (induced) signals, and the emergent morphogenetic properties associated with collective cellular behavior in any given system. Our short-term (1-2 yr) goal is to build a *directed computer animation* of vertebrate eye development that is biologically-based and mathematically-driven. The research prototype will be validated and refined against standardized tests for subsequent expansion to other embryonic systems (3-5 yr).

The first task is to structure a graphical model based on developmental ontology of the embryonic eye. In mouse, gestation days (GD) 7 to 11 encompass the sequence of events spanning vulnerability for congenital eye malformations. This is a good place to start (see diagram below). The eye originates from head surface ectoderm, forebrain neuroectoderm, and associated mesenchyme through a series of reciprocal tissue interactions that are controlled by genetic signals and responses. The master switch is *Pax6*, a pair-rule homeobox gene that sets up local responses to regional signals such as sonic hedgehog (SHH), bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs). An outgrowth of the forebrain known as the ‘optic vesicle’ is driven by SHH elaborated from the chordamesoderm. Acting through orthodenticle (*Otx2*) and retinal homeobox (*Rx1*) genes, the optic

vesicle secretes BMP4 and, as it approaches the surface ectoderm, dramatically up-regulates *Sox2* expression. Once PAX6 and SOX2 are together in the same cell the lens placode (LP) invaginates and produces FGF, which in turn acts back on the neural retina (NR) to simulate mitosis and folding. Meanwhile BMP7 released from the extraocular mesenchyme specifies the pigmented retina (RPE) through *Mitf*. Formalizing the associative relationships between an anatomical structure and its spatial location, functional system and chronological stage in the embryo requires hierarchical information. Such detailed information is handled through 'ontologies' that link facts as a triad of related terms. Two of the best known ontology languages, namely OBO (Open Biomedical Ontology) and OWL (OWL Web Ontology Language), have been used to write developmental ontologies for Theiler Stages [Bard, 2007] and Carnegie Stages [Hunter et al. 2007] in mice and humans, respectively (http://obofoundry.org/).



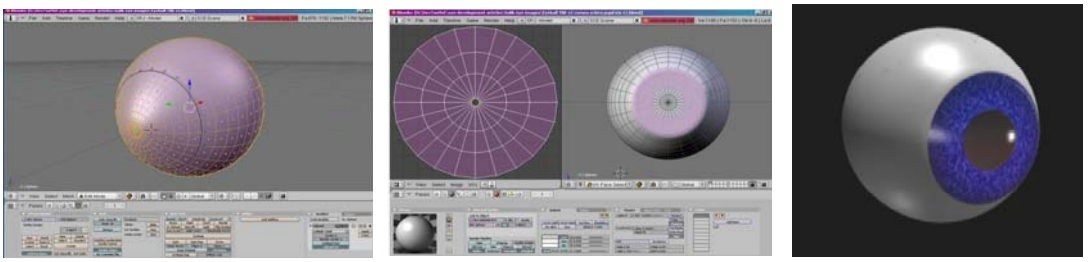
Developmental ontology for the eye primordium of the mouse. Overview across Theiler Stages (TS) corresponding to optic pit (TS12-13), optic vesicle (TS14-15), lens invagination (TS16-17), and optic cup (TS18). The median neuraxis is organized by the chordamesoderm (CM). The optic sulcus is marked by the optic pits (OP) as these invaginate into the primary mesenchyme (grey) to approach competent surface ectoderm (presumptive lens ectoderm, PLE). BMP emanating from the central bulge (prospective neural retina, PNR) induces lens placode (LP) formation. The LP in turn stimulates the neural retina (NR) through FGF release. In-pocketing of the lens pit and its detachment as the lens vesicle (LV) from surface ectoderm (future cornea) is associated with classical morphogenetic cell death. Sites of differentiation of retinal pigment epithelium (RPE), ciliary body and iris (C/I) are shown. Morphogenesis of the ventral optic stalk (VOS) results in closure of the optic fissure, similar to the neural tube, to complete the posterior chamber. The VOS forms a pathway for optic nerve outgrowth as retinal layers organize and differentiate GD11+ [series adapted from Chow and Lang, 2001]. The developmental ontology annotations are based on the EMAP system [Bard, 2007] and colored to match the anatomical representation.

The Edinburgh Mouse Atlas Project (http://genex.hgu.mrc.ac.uk/intro.html) is mapping successive stages of mouse embryonic development to catalogue gene expression domains as voxel images with ~7-micron resolution [Baldock et al. 1992]. We applied EMAP scaling to scanning electron micrographs (SEMs) of mouse eye development (http://www.med.unc.edu/embryo_images/unit-

welcome/welcome_htms/contents.htm). The computed width of 23 cells across the PLE field was consistent with an estimate of ~20-25 cells determined by counting PAX6-positive nuclei. We then modeled the development of axis trajectories using SigmaPlot 2001 for Windows version 7.0 (SPSS Inc.). The best fit was a simple exponential growth model, y = a*b^x, for the dimension (y) at some point in time (x). From the initial model we can later fill in real values from systematic analysis of normal and abnormal ocular phenotypes using other sources of morphometric data from online atlases or micro-PET / micro-CT imaging cooperative agreements.

Parameter	Coefficient	Standard error	P	R^2 adj R^2 sqr
eye width				
variable a	87.8643	18.4709	0.0089	0.98156361
variable b	1.0279	0.0034	< 0.0001	0.95433389
eye depth				
variable a	77.3446	22.6413	0.0269	0.96849494
variable b	1.0282	0.0048	< 0.0001	0.92247805
lens width				
variable a	87.7712	5.0620	< 0.0001	0.99368307
variable b	1.0172	0.0010	< 0.0001	0.98425755
lens depth				
variable a	0.7208	0.1232	0.0043	0.99969671
variable b	1.0857	0.026	< 0.0001	0.99924413

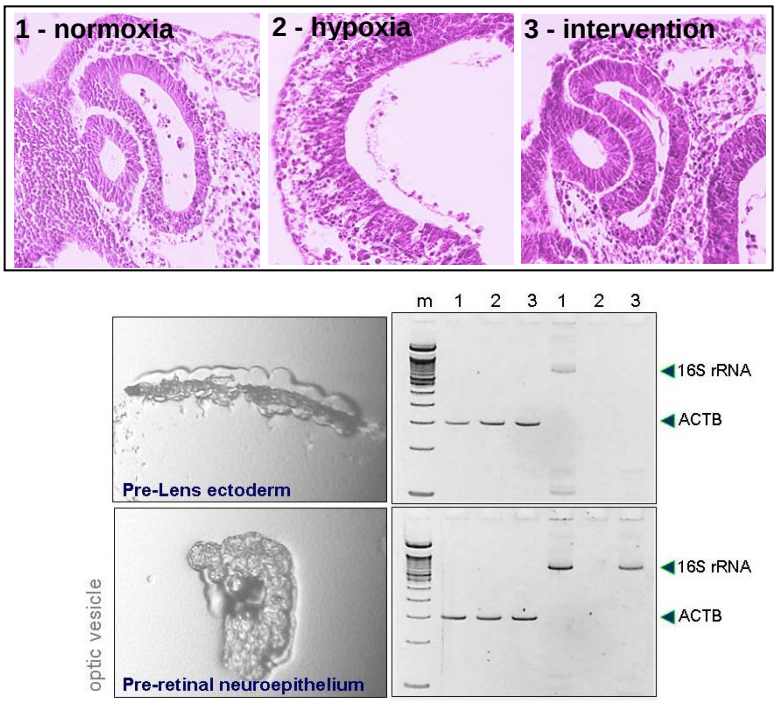
Visualization will use open-source software (**Blender**foundation, http://www.blender.org). Blender's mesh and render commands make it practical to animate ocular development along the growth equations using a primitive mesh of base objects - cuboidal cells in this case, with a modifier lattice applied to change the objects and mesh topology according to the formalized developmental program. Our short-term goal is to complete the directed animation schema, first for the lens vesicle and then for the optic cup. To do this a primitive mesh representing the optic cup (GD11) will be designed and packaged into the optic field (GD8). Its evolution will then be directed by a control-lattice that would in essence deform the optic field to evolve the lens and retina. Fitting coordinates (pixels) and values (spin) of the control-lattice to morphometric trajectories solved by the computational model can solve the visual eye model.



The computational eye model can be solved using agent-based and cellular automata simulators. Hybrid cellular automata (CA) simulators have been used to integrate collective cell behavior into models of spatial patterning and growth during limb morphogenesis [Izaguirre et al. 2004; Cickovski et al. 2005; Robertson et al. 2007]. Several open-source software applications are available. CompuCell3D (http://compucell.sourceforge.net) is one simulation environment that has three interactive modules: a cellular Potts model (CPM) that is basically the CA simulator, a Reaction-Diffusion (RD) module that overlays signaling gradients, and a state-type (ST) module that assigns parameter set (type), specific values (state), and rules to govern state-change and type-change [Izaguirre et al. 2004; Cickovski et al. 2005]. Other hybrid CA simulators have been used to integrate subcellular information into continuous and discrete models of spatial patterning and growth based on microheterogeneties of cell-matrix interactions using the matrix components and cell receptors as 'agents' [Robertson et al. 2007]. A simple and accessible program for creating such agent-based models (ABM) is available from NetLogo (http://ccl.northwestern.edu/netlogo/). In these hybrid models the CA is updated using information collected by internal parameters (genetic) and the immediate neighborhood (environment) [Dormann and Deutsch, 2002]. Projecting the coordinates onto Blender will associate an integer index with each lattice site (voxel) to identify the spatial extent and location of each cell at any instant.

The cell behavior data needed to construct our cell-based model will initially come from the literature but will likely require new experiments because the model assumes *a priori* knowledge of the shape of the eye as a function of time. Successful simulators will require a good deal of data for parameter assignment and, since these data may be less complete for ocular teratogenesis we can run the analytical model over the short-term with limited experimental details and simulated perturbations to define a minimal set of behaviors to judge outcome. A parameter sweep can inform of the combinations of cellular consequences that lead to a particular phenotype based on the model's initial conditions, agents and ingredients, and rule specification. Since genes and their products determine the biological properties of cells during morphogenesis, useful computational models of eye development must integrate the cell-based responses with the genetic circuitry that underlies the key signals and responses and coordinates metabolic differentiation.

More explicity, the mechanistic modules that would comprise the eye model could have a commonality with other efforts at the EPA addressing a mitochondrial subcomponent. Our research over the years has pointed to a mitochondrial-based mode of action for several ocular teratogens. For example, development from optic pit to optic cup is associated with switchover from anaerobic (glycolytic) to aerobic (oxidative) metabolism [Ibrahim et al. 1998]. Exposing GD9 mouse embryos to venous oxygen (5% oxygen), which is preferred on GD8, for 2h on GD9 arrests eye development at the pre-placodal lens stage. Normal development can be recovered with PK11195, a mitochondrial drug.



Selective susceptibility of the eye primordium to oxygen variance. Day 9 mouse embryos at the optic vesicle stage cultured 24h to the optic cup stage in a conventional whole embryo culture system. LEFT: normal lens vesicle induction under 21% (arterial) oxygen. MIDDLE: when embryos received 5% (venous) oxygen for the 1st 2h, then recovered to 21% oxygen the lens failed to develop in a significant number of cases. RIGHT: the mitochondrial drug PK11195 blocked the adverse response to transitory hypoxia [O'Hara et al. 2003].

Cellular microgenomics. Laser capture microdissection of presumptive lens ectoderm (PLE) and prospective neural retina (PNR) at the 2h time-point followed by RNA amplification and PCR revealed the effect on the mitochondrial 16S rRNA biomarker.

5. What other components of EPA or outside organizations are involved?

Collaborators from NCCT: This project will initially operate within the Biological Modeling Group and thus is likely to benefit from the input of members from that group. The project will also align closely with the information management aspect of the ToxCast™ Research Program and with systems modeling in the Virtual Liver Project.

Other EPA linkages: The project will develop novel interactions with the National Health Environmental Effects Research Laboratory (NHEERL), Reproductive Toxicology Division for expertise in experimental embryology - teratology (Chris Lau, Sid Hunter, John Rogers) and the USEPA Office of Pesticide Programs (Liz Mendez, Elissa Reaves, Vickie Dellarco), and NCEA (Sue Euling, Sue Makris) for expertise in regulatory toxicology and developmental toxicology.

Outside partners:

- Catherine Blake School of Information and Library Science, University of North Carolina (cablake@email.unc.edu) and Eric Rouchka (eric.rouchka@louisville.ed), Department of Computer Sciences and Computer Engineering at the University of Louisville, are potential partners in the automation of logic-based text-mining.

- Dave Wise of the Developmental and Reproductive Toxicology group at Merck Research Laboratories (LD_wise@merck.com), who was the originator of the DevTox terminology atlas, and Jochen Buschmann at the Fraunhofer Institute (buschmann@item.fraunhofer.de) who is now overseeing the atlas.

- Kathy Sulik, Department of Anatomy and Cell Biology at the University of North Carolina (mouse@med.unc.edu) and Richard Baldock, Senior Scientist at the Medical Research Council, Human Genetics Unit, Western General Hospital Edinburgh UK (Richard.Baldock@hgu.mrc.ac.uk), are potential partners in morphometry.

- Beth Julien, International Life Sciences Institute (bjulien@ilsi.org) and Ray Tice, Staff Scientist at the NIEHS National Toxicology Program (tice@niehs.nih.gov) are potential partners in the traditional developmental toxicology database.

- Aldert Piersma, Director of Reproductive and Developmental Toxicology at the RIVM, Bilthoven The Netherlands (ah.piersma@rivm.nl) and Ken Ramos, Director of the Center for Environmental Gneomics and Integrative Biology at the University of Louisville (kenneth.ramos@louisville.edu), are potential partners in data generation.

- Greg Rempala, Department of Mathematics (g.rempala@louisville.edu) and Trevor Cickovski, Department of Computer Science and Engineering, University of Notre Dame and innovator of CompuCell3D (tcickovs@nd.edu), are potential partners in applied mathematics and programming.

6. How is data management being achieved?

BDSM will provide the knowledge management for *v-Embryo.* This includes routine: (a) consolidation of communal data and metadata relevant to developmental health and disease; (b) interactions with current builds of national databases and data repositories; (c) user-friendly web portals specifically designed for data tracking provenance; (d) efficient algorithms for cross-species annotation of symbolic gene annotations that reverse-engineers the NCBI sequence homology-based annotations into corresponding homologues and orthologues; (e) programming retrieval capability that allows highly personalized queries across experiments to facilitate secondary analysis; and (f) data formats made interoperable with the suite of commercial and free analysis software for data merging, phenetic clustering, chromosomal mapping, gene ontology classification, pathway evaluation, and network identification. The BDSM portal will be managed in ACToR (R Judson), and knowledge base development will be managed using open source Sesame (I Shah).

7. What are appropriate measures of success?

Short-term outcomes (1-2 yr):

- formulate research plan and white paper for *v-Embryo*
- enlist collaborators from NCCT
- establish EPA linkages with NHEERL, OPP and NCEA
- recruit postdoctoral fellow and undergraduate/graduate student
- establish outside partners (UNC, RENCI, ILSI, DevTox, EMAP, JAX, UL, RIVM, NTP)
- migrate BDSM to EPA and link to ACToR
- directed (computational) and ontology (visual) model for simulating morphogenesis of the eye
- submit genetic circuitry of eye development to the BioTapestry library
- attempt recapitulation of known ocular phenotypes, using simulated data

Long-term outcomes (3-5 yr):

- validate recapitulation of ocular phenotypes, using new empirical data
- address mitochondrial mechanisms in developmental toxicity
- extend knowledge-discovery and computer-simulation to other developing systems
- expand BDSM resources and tools for integrative database management
- build *Regulatory Toxicology Support System* (RTSS) module for BDSM
- establish collaborative research program for innovation (RFA) and validation (CRADA)
- assess project goals for predictive modeling and steps to regulatory acceptance
- seek renewable funding ($500K/yr) via interagency agreements with NICHD, NIEHS, NEI, NIAAA)
- motivate the use of these tools and approaches by the broader scientific community

REFERENCES

Baldock R, Bard J, Kaufman M and Davidson D (1992) A real mouse for your computer. BioEssays 14: 501-503.

Bard J (2007) Systems developmental biology: the use of ontologies in annotating models and in identifying gene function within and across species. Mamm Genome 18: 402-411

Bremer S, Pellizzer C, Hoffmann S, Seidle T and Hartung T (2007) The development of new concepts for assessing reproductive toxicity applicable to large scale toxicological programmes. Curr Pharm Des. 13: 3047-3058

Charlap JC, Donahue RJ and Knudsen TB (2003) Exposure-disease continuum for 2-chloro-2'-deoxyadenosine, a prototype ocular teratogen. 3. Intervention with PK11195. Birth Defects Res (A) 67: 108-115

Chow RL and Lang RA (2001) Early eye development in vertebrates. Annu Rev Cell Dev Biol 17: 255-296

Cickovski TM, Huang C, Chaturvedi R, Glimm T, Hentschel HGE, Alber MS, Glazier JA, Newman SA and Izaguirre JA (2005) A framework for three-dimensional simulation of morphogenesis. IEEE/ACM Trans Comput Biol Bioinfor 2: 1-15

Davidson EH and Erwin DH (2006) Gene regulatory networks and the evolution of animal body plans. Science 311: 796-800

Dormann S and Deutsch A (2002) Modeling of self-organized avascular tumor growth with a hybrid cellular automaton. *In silico* Biology 2: 0035

FitzPatrick DR and van Heyningen (2005) Developmental eye disorders. Curr Opin Gen Devel 15: 348-353

Green ML, Singh AV, Zhang Y, Nemeth KA, Sulik KK and Knudsen TB (2007) Reprogramming of genetic networks during initiation of the fetal alcohol syndrome. Devel Dynam 236: 613-631

Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A, Cuellar AA, Dronov S, Gilles ED, Ginkel M, Gor V, Goryanin II, Hedley WJ, Hodgman TC, Hofmeyr JH, Hunter PJ, Juty NS, Kasberger JL, Kremling A, Kummer U, Le Novere N, Loew LM, Lucio D, Mendes P, Minch E, Mjolsness ED, Nakayama Y, Nelson MR, Nielsen PF, Sakurada T, Schaff JC, Shapiro BE, Shimizu TS, Spence HD, Stelling J, Takahashi K, Tomita M, Wagner J and Wang J (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19: 524-531

Hunter A, Kaufman MH, McKay A, Baldock, R, Simmen MW and Bard JBL (2007) An ontology of human developmental anatomy. J Anat 203: 347-355

Ibrahim MM, Razmara M, Nguyen D, Donahue RJ, Wubah JA and Knudsen TB (1998) Altered expression of mitochondrial 16S ribosomal RNA in p53-deficient mouse embryos revealed by differential display. Biochem Biophys Acta 1403: 254-264

Izaguirre JA, Chaturvedi R, Huang C, Cickovski T, Coffland J, Thomas G, Forgacs G, Alber M, Hentschel G, Newman SA and Glazier JA (2004) CompuCell, a multi-model framework for simulation of morphogenesis. Bioinformatics 20: 1129-1137

Knudsen TB and Green ML (2004) Response characteristics of the mitochondrial DNA genome in developmental health and disease. Birth Defects Res (C) 72: 313-329

Knudsen KB, Singh AV and Knudsen TB (2005) Data input module for Birth Defects Systems Manager. Reprod Tox 20: 369-375

Longabaugh WJR, Davidson EH and Bolouri H (2005) Computational representation of developmental genetic regulatory networks. Dev Biol 283: 1-16

National Research Council, Committee on Toxicity and Assessment of Environmental Agents. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy (2007). National Academies Press (http://www.nap.edu/catalog/11970.html)

Nemeth KA, Singh AV and Knudsen TB (2005) Searching for biomarkers of developmental toxicity with microarrays: normal eye morphogenesis in rodent embryos. Toxicol Appl Pharmacol 206: 219-228

Olson EN (2006) Gene regulatory networks in the evolution and development of the heart. Science 313: 1922-1927

O'Hara MF, Nibbio BJ, Craig RC, Nemeth KR, Charlap JH and Knudsen TB (2003) Mitochondrial benzodiazepine receptors regulate oxygen homeostasis in the early mouse embryo. Repro Tox 17: 365-375

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504

Singh AV, Knudsen KB and Knudsen TB (2005) Computational systems analysis of developmental toxicity: design, development and implementation of a birth defects systems manager (BDSM). Reprod Tox 19: 421-439

Singh AV, Knudsen KB and Knudsen TB (2007a) Integrative Analysis of the mouse embryonic transcriptome. Bioinformation 1: 24-30

Singh AV, Rouhka EC, Rempala GA, Bastian CD and Knudsen TB (2007b) Integrative database management for mouse development: systems and concepts. Birth Defects Res (C): 81: 1-19

Robertson SH, Smith CK, Langhans AL, McLinden SE, Oberhardt MA, Jakab KR, Dzamba B, DeSimone DW, Papin JA and Peirce SM (2007) Multiscale computational analysis of Xenopus laevis morphogenesis reveals key insights of systems-level behavior. BMC Systems Biology (in press) doi:10.1186/1752-0509-1-46

Wubah JA, Setzer RW, Lau C, Charlap JH and Knudsen TB (2001) Exposure-disease continuum for 2-chloro-2'-deoxyadenosine, a prototype ocular teratogen. I. Dose-response analysis. Teratology 64: 154-169